Editorial Comments

THE "CAUSE" OF CANCER

The meeting of the British Medical Association at Bath, which was held there from July 17th to 25th, was notable for the large number of members who took part, for the lavish hospitality offered by the ancient city and for the announcement of the discovery by Dr. W. E. Gye and Mr. J. E. Barnard, F.R.S., of the two causal factors responsible for the growth of malignant tumours.

The announcement was made on July 22nd at the morning session of the section of pathology under the Chairmanship of Professor Ledingham, C.M.G., F.R.S., Chief Bacteriologist at the Lister Institute. The meeting took place in the Museum and the Literary and Scientific Institute which on that very hot morning was crowded to its utmost capacity. The title of the discussion was "Filter-passing Viruses."

The *Lancet* of July 18th contained two papers: "The ætiology of malignant new growths," by Dr. Gye, and, "The microscopical examination of filterable viruses associated with malignant new growths," by J. E. Barnard, F.R.S., of which the communications at Bath were a résumé. The two papers are in fact to some extent independent; the former dealing with the biology of the problem, the latter with its microscopy. The logical chain of evidence appears complete that "cancer" is the outcome of an infective, filterable, living virus operating in a tissue which has developed some sort of intrinsic specific receptivity. The physicist's part has been to devise an optical method of rendering visible this parasite which is invisible in the "ordinary" microscope, and also of photographing it.

The present research begins where Dr. Peyton Rous of the Rockefeller Foundation left off.

In 1911 Dr. Rous announced that he had been able to transfer a sarcoma of the fowl (Plymouth Rock) from chicken to chicken by all of the following procedures: Inoculating dead tumour cells, or tumour cells killed by drying or killed by 50 per cent. glycerol. Most important of all, Rous discovered that by inoculating a chicken with the cell-free filtrate (through a Berkefeld filter) of an extract of tumour, the sarcoma in question would be produced.

Further, Rous and his co-workers showed in a series of papers published between 1911 and 1916 that the living infective agent which had passed through the pores of a Chamberland filter was killed or rendered inert by a temperature of 55°C. applied for fifteen minutes, and by treatment with chloroform, with toluene or with phenol. (0.5 per cent.).

The Rous tumour itself retains its infectivity even when immersed in glycerol.

The problem at the conclusion of Rous's work was to determine the nature of the filterable "agent" which Rous would not call a "virus" because he had not succeeded in cultivating it outside the body.

Dr. Gye as the result of his investigations believes the agent or "cause" of cancer to be an intra-cellular parasite, but a parasite so minute that it belongs to an order of dimension which is quite unfamiliar to all "ordinary" microscopists.

Mr. Barnard gives us some idea of the extreme minuteness of this latest identified organism or "virus", if "organism" connotes too much. The micron (μ) is one thousandth 0.001 of a millimetre. The diameter of a human erythrocyte is seven microns (0.007 mm.), that of a small micrococcus is one micron (0.001 mm.); the magnitude of a single cancer germ is 0.075 of a micron (0.000,075 mm.), or 100,000 times as small as a human red blood corpuscle.

It will be most convenient to summarize Dr. Gye's conclusions.

1. He believes that all malignant neoplasms contain an intracellular virus which can be extracted by a solution, e.g., Ringer's and made to pass through a Berkefeld filter.

This applies both to sarcomas and carcinomas of fowls, mice, rats, dogs and men.

2. The virus has been cultivated in vitro.

3. The virus *washed free* from all adherent material, when injected into a living animal does not produce a tumour, it does not even produce a visible lesion.

4. When an extract of the tumour made virus-

free and therefore non-infective is injected into an animal at the same time as the virus itself is injected, a cancerous growth develops.

This second factor Dr. Gye calls the "specific factor"; and he believes it destroys the natural resistance of the body-cells to the inroads of the virus. He thinks it to be of a chemical nature.

5. The specific or chemical factor injected by itself produces no tumour.

6. There is no species-specificity in the case of the virus, for the virus of one species will produce a tumour in any other species but—

7. The specific (chemical) factor shows the strictest species-specificity and even tissue-specificity.

Thus a cancer in a mouse can be produced by the virus from any species, e.g., fowl, but only when the second or chemical factor derived from mouse-tumour has also been injected. We note, then, the non-specificity as regards species of the cancer virus. It would appear that the virus is omnipresent and equally responsible for all the forms of cancer (sarcoma and carcinoma). Why we do not all get cancer all the time is because the second (chemical) factor is not only not present all the time, but is highly specific when it is present. In all probability each tissue has its own chemical factor.

Dr. Gye uses the term "primary culture" to mean the placing of a fragment of the fowl or other sarcoma in a tube of culture-medium (broth), the infectivity of which the addition of rabbit-serum is found to increase. It has also been discovered that the infectivity is increased or prolonged by keeping the culture under anærobic conditions. Oxygen is inimical to the virus; a very interesting point. As might be expected, the infectivity varies directly with the size of the tumour taken; and as the upper layers of the supernatant fluid are the most infective, it would seem probable that something diffuses out of the tumour and invades the upper layers.

Perhaps the most important portion of Dr. Gye's work has been the cultivation of the virus *in vitro*. He found that by prolonged centrifugalization of the clear fluid above a primary culture, the virus could practically all be thrown down, for the injection of some of the supernatant fluid produced a very small tumour, whereas, injection of the deposit gave rise to a large neoplasm. The medium most useful for cultivation *in vitro* contains rabbit- serum and potassium chloride to which a fragment of a 12- to 16-day-old chick embryo has been added. The first "sub-culture" is a tube of the medium just described to which a loopful of "primary culture" has been added. This tube is incubated anærobically at 36°C. for four days. The second sub-culture is established by a similar procedure; the dilution with each sub-culture is at least a thousand times. In the fifth sub-culture the original inoculum has been diluted $10.^{15th}$ time. If tumours are produced with sub-cultures beyond the fifth, we may be certain that growth of the virus has taken place.

Such tumours are so produced, and therefore we conclude that growth in vitro has certainly occurred.

The main experimental facts may now be summarized:—

1. Any virus obtained from any neoplasm injected by itself into any kind of animal has no effect.

2. Any "specific substance" from any neoplasm injected into any kind of animal has no effect; whereas,

3. The virus of mouse-carcinoma along with the specific substance from fowl-sarcoma injected into a fowl will give rise to sarcoma, but into mice will have no effect.

4. Human carcinoma virus along with the specific substance from fowl-sarcoma injected into fowls will give sarcoma, but into mice nothing.

Thus while the "germ theory" of cancer is established, it is highly important to know that even the living virus by itself will not infect: there must be the specific receptivity or concomitant internal factor. Dr. Gye rightly regards this second factor—the chemical, specific one—as of immense importance.

He and Dr. Cramer showed some years ago that the bacteria, for instance, of gas-gangrene, or of tetanus by themselves are not infective; it required some chemical substance such as colloidal silicic acid to produce that lowering of the resistance which permitted of a lethal result. This lowering of resistance these authors have called "defence-rupture" or "kataphylaxis." Such tissue resistance or physiological insusceptibility to an extrinsic agent, Fraser Harris in 1908* had cited as one of the examples of "protoplasmic or functional inertia."

*The Functional Inertia of Living Matter, Churchill, 1908.

Dr. Gye has for the first time in regard to cancer actually proceeded from the vague to the concrete to the extent of isolating the chemical factor responsible for the rupture in the defences which permits of the virus beginning its work. It should be stated that a few months ago, Dr. Coley of New York, in reviewing the evidence for the parasitic origin of cancer, definitely suggested that in order to attack the tissues and induce them to produce a malignant growth some kataphylactic factor as postulated by Cramer and Gye was required.

Mr. Barnard in his paper on "The microscopical examination of filterable viruses associated with malignant new growths", begins by reminding us that late in the nineteenth century it was recognized that the theoretical limits of resolution of details by the microscope had been approached in actual practice.

The visibility and discreteness of the parts of a minute object depend on the intensity of the illumination and on the resolving power of the objective quite as much as on the magnification. There are only two methods of illuminating an object: One to transmit the light through the object which must not, therefore, be perfectly transparent, and the other to cause the light to fall upon the object which may reflect it, diffract it or scatter it. In the latter case the ground or field must be dark. Objects or elements of structure that are less than 0.25 μ (0.00,025 mm.) in diameter cannot be resolved into visible parts by any microscopical apparatus in present use.

Any new apparatus which can "resolve" objects of less than this diameter may be called an "ultra-microscope". It is an instrument of this sort which Mr. Barnard has devised. He writes, "By the use of ultra-violet light it is possible to obtain a real image of a small body provided a short enough wave-length is used." The source of light is a quartz-mercury-vapour lamp of the non-vacuum type; the observer must work in a semi-darkened room because his eye must be dark-adapted. Mr. Barnard uses light of 257 $\mu\mu$; and an objective made of quartz. The magnifications employed are 1850 and 2200 diameters. The paper in the *Lancet* of July 18th has photomicrographs of the virus as seen both with transmitted light and in dark-ground illumination.

One of the most important sections of Mr. Barnard's communication is that dealing with the artificial cultivation *in vitro* of the filter-passing cancer virus and of other similar viruses. His method was to allow serum-agar to flow over rectangular glass slips in a test-tube and to inoculate these with the filtered virus. After incubation, the film was covered with a glass or quartz cover-slip which permitted of cedar wood oil being used with the immersion lens. Fluid cultures from malignant growths are known always to remain clear; grown on solid media they are so minute as to be observable only by high power lenses. The colonies which are quite invisible to the naked eye, can be viewed by dark-ground illumination or photographed by light of very short wave-length.

Mr. Barnard believing, however, that ultra-violet light is destructive of the virus, is compelled to use as short exposure as possible. We are now assured that several other diseases are probably due to filter-passers. Barnard has already photographed the virus of bovine pleuro-pneumonia; and at the same meeting as that at which he and Dr. Gye spoke, Dr. Mervyn Gordon read a paper on the virus of smallpox as a filter-passer. Besides the poison of typhus, that of the distemper of dogs and that of encephalitis lethargica are now believed to belong to this group; influenza too is probably of the same character.

Dr. Gye's own name is William Ewart Bullock: he changed it to Gye on his marriage with a lady of that name (the G is soft). He was born in Derbyshire about forty years ago. "Billy" Bullock, the son of a railway signalman, was himself for a short time a railway porter. After studying at University College, Nottingham, and taking the degree B.Sc. of London, Gye went to Edinburgh to graduate in medicine. He obtained his M.D. as well as two gold medals during his course. Dr. Gye served in France and in Italy during the Great War. It is said that it was the fact that his mother died of cancer which turned his thoughts towards the problem of the origin of malignant growths. His success in the solution of that problem is a gratifying vindication of the national usefulness of the state-aided Medical Research Council.

Mr. Barnard is an older man and not a graduate in medicine. He is a F.R.S. and probably the most expert microscopist in Europe. He is Lecturer in Microscopy at King's College, London, and Director of the Department of Applied Optics, at the Institute for Medical Re-

search, Hampstead. As a child he was given a toy microscope by his father, and this so aroused his interest in the life of the invisible that he occupied all his leisure in studying microscopy and microphotography; to what purpose we all now know. A few days ago Dr. Gye and Mr. Barnard were received by H. M. the King at Buckingham Palace.

D. FRASER HARRIS

London, August 1st.

CANCER IN CANADA

There are few problems of the day which are receiving more concentrated attention than that of the causation and control of cancer; there certainly is no one factor which is a more serious menace to the health of the people. Therefore, the warnings which we are given by statistical studies of the question must not be allowed to fall on unheeding ears. One such study has recently been made by Fred. L. Hoffman, L.L.D.,* and apart from its intrinsic excellence, there is an added interest in the fact that the analysis applies to Canada alone. The value of the study is heightened, as Dr. Hoffman indicates, by the fact that there has been in Canada great improvement in late years in the methods of registration, classification, and analysis of vital statistics.

His observations deal with sixteen Canadian cities, which in 1921 had a combined census population of 2,200,000, and the returns for these cities are limited to the last fifteen years. By way of introduction, however, he shows that in twenty years up to 1924, the deaths from cancer had increased in the first decade at the rate of 14.5 per 100,000, whilst in the last decade the increase was 20.4. The average total mortality for the first decade was 60.1, and for the second decade it was 80.1 . He had previously shown that the annual increase in the cancer death-rate of the United States was about two and a half per cent., corresponding quite closely to the 3.1 annual increase shown by the Province of Ontario.

Such figures, however, must be analyzed as regards the various organs and parts of the body affected, and also the different types of the disease. The Province of Ontario provides statistics which make this further analysis possible, and some interesting facts are brought out. Cancer of the buccal cavity, for example, the mortality from which in 1914 prevailed at the rate of 4.1 per 100,000, was 4.4 in 1923, an increase of no great significance. Cancer of the breast, however, shows an increase from 4.5 in 1914 to 9.2 in 1923; of the female generative organs, an increase from 5.2 in 1914 to 9.4 in 1923. There is a particularly suggestive increase in the mortality from cancer of the stomach, which has increased, though with intervening fluctuations, from 22.8 in 1914 to 31.0 in 1923.

Dr. Hoffman decides therefore that a marked increase has taken place during the period under observation. "It lies outside the realm of reasonable proportion that this particular increase, as indicated by statistics, should not be in approximate formity to the actual facts of the situation. The increase conforms in a general way to the data elsewhere dealt with for both the United States and Canada."

The number of cancer death annually for the whole of Canada has been conclusively established as about 6,000. The best measure available at the moment of the "cancer trend" in Canada, is a consolidated return of certain Canadian cities, beginning with ten cities in 1910 and reaching sixteen in 1923, diminishing to fourteen in 1924. The ominous rise in the death rate as shown by these figures is best appreciated from the tables given in Dr. Hoffman's paper, but the total increase is impressive enough. The rate in 1911 was 58.6; in 1924 it was 98.1 per 100,000, and "for all practical purposes this rate corresponds to the combined cancer death-rate of the United States."

Only a few points brought out in the analysis of these figures can be given here. In the first five years of the period only one city had a rate of over 100, (Halifax, 100.9). In the second five year period three cities showed a rate in excess of 100, namely, Halifax, Vancouver, and Victoria, and in the third five year period there were added to these cities in this class, St. John, Hamilton and Toronto.

Montreal, Ottawa, and Quebec, show relatively low rates in a period in which other cities show large increases, and Dr. Hoffman believes that the French Canadian population are less liable to cancer than the population of British origin, a point which he feels strongly to be in need of investigation. He holds also that the Indian population is unquestionably less liable to can-

*The Public Health Journal, Toronto, June, 1925, No. 6.